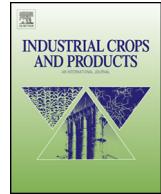




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Simultaneous detoxification, saccharification, and ethanol fermentation of weak-acid hydrolyzates



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ABSTRACT

Lignocellulosic feedstocks can be prepared for ethanol fermentation by pre-treatment with a dilute mineral acid catalyst that hydrolyzes the hemicellulose and opens up the plant cell wall fibers for subsequent enzymatic saccharification. The acid catalyzed reaction scheme is sequential whereby released monosaccharides are further degraded to furans and other chemicals that are inhibitory to the next fermentation step. This work evaluated the use of agricultural residue (flax shive) as starting material for making activated biochar to adsorb these degradation products. Results show that both furfural and hydroxymethylfurfural (HMF) are adsorbed by steam-activated biochar prepared from flax shive. Decontamination of the hydrolyzate significantly improved the fermentation behavior by *Saccharomyces cerevisiae* yeast, including significantly reducing the lag phase of the fermentation, when the amount of biochar added to the fermentation broth was 2.5% (w/v). No negative effects were noted from addition of activated char to the process.

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1. Introduction

Lignocelluloses have been promoted for many years as an attractive resource for production of biofuels, chemicals, and bioproducts. Fermentation of biomass into ethanol or other products typically requires first hydrolyzing the carbohydrates into fermentable sugars. There are several competing methods of hydrolysis but one of the most widely accepted method involves pretreating biomass with a dilute mineral acid catalyst that hydrolyzes the hemicellulose to oligomers and monosaccharides (Hahn-Hägerdal et al., 2006). After pretreatment, cellulose is converted into monosaccharides using cellulases and other related enzymes for subsequent fermentation to biofuels. However during pretreatment, sugar and lignin degradation products are produced that are inhibitory to many ethanol-producing microorganisms and, if not removed, will either prevent fermentation or result in a prolonged lag phase and reduced product yield (Boyer et al., 1992). The main inhibitors are furfural (mainly from xylose degradation) and hydroxymethylfurfural (HMF) (mainly from glucose degradation). The minimum inhibitory levels vary from microorganism to microorganism but are generally below 1 g/L (Weil et al., 2002; Mussatto and Roberto,

2004), and furfural is more toxic than HMF. Recently, Mussatto and Roberto (2004) and et al. (2008) Huang reviewed options for detoxifying these hydrolyzates. Many methods that have been studied in the past focused on pH adjustment in combination with activated charcoal treatment but the charcoal was removed from the hydrolyzate prior to fermentation (Gong et al., 1993; Dominguez et al., 1996; Parajó et al., 1996; Alves et al., 1998; Silva et al., 1998; Mussatto and Roberto, 2001). Even more recent studies that utilize sorption schemes to remove fermentation inhibitors suggest segregating the detoxification and the fermentation (Hodge et al., 2009; Ranjan et al., 2009; Zhang et al., 2011; Lee et al., 2011). Even biological removal techniques (microbial or enzymatical) to remove the inhibitors have been studied (Parawira and Tekere, 2011).

Most previous studies applied commercially available activated carbons that were manufactured from coal. However, activated carbons can also be generated using sustainable biomass feedstocks. The production of carbons from fibrous lignocellulosic materials involves pyrolysis under an inert atmosphere before it is activated (Lima et al., 2004). Steam activation is the most common method of producing activated carbons, but acid activation, or carbon dioxide activation has also been used (Johns et al., 1999; Toles et al., 2000a,b). One source of biomass previously proposed for production of activated carbon is flax shive (Cox et al., 1999).

Flax (*Linum usitatissimum* L.) is a source for fiber (for linen textiles) and oil (linseed oil) for domestic and industrial uses (Berglund, 2002; Domier, 1997; Van Sumere, 1992). In order to obtain the

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fiber (which is contained in the stem) the material is retted, which separates the fibers from the other parts of the stem. The most common type of retting is dew-retting (Van Sumere, 1992). The 2007 flax seed production was 5.9 million bushels (150,000 metric ton) in the U.S. (USDA, 2008) and 989,000 ton in Canada (AAFC, 2008). The seeds only constitute a small part of the plant and the shive byproduct yield is approximately 1.88 kg shive/kg of seed. Thus, the potential amount of fiber-free flax shive available in North America can be estimated at 2.1 million ton annually (Klasson et al., 2009).

In this manuscript, we report on a process that is built on simultaneous saccharification, detoxification, and fermentation. In this process, activated biochar made from flax shive is added to the hydrolyzate prior to fermentation. The fermentation progresses in the presence of the activated biochar. Prior work often considered sorbent recycle (or inhibitor recovery) as an important part of the process and separated the char prior to fermentation. However, the added separation step makes the process more complex, adds additional expensive equipment requirements, and may in the final analysis not prove cost effective. Considering that the spent microbial cells after fermentation usually contain transgenic organisms along with residual lignin and will be combusted, additional carbon-like materials in this matrix will likely increase the value (e.g., fuel value) of this waste product. In addition, sustainability is enhanced by using activated carbons from biomass, which have previously been found to have good adsorption capacity for the fermentation inhibitors furfural and HMF (Klasson et al., 2011).

2. Materials and methods

Several different experiments were performed as part of this study. The first set of experiments was designed to see how well steam activated biochar would remove the fermentation inhibitors furfural and HMF in a lignocellulosic acid hydrolysis liquor. The second set of experiments took the process a step further by determining how well the biochar removed the fermentation inhibitors from hydrolyzates that had undergone both acid pretreatment and enzyme hydrolysis. The third and fourth set of experiments focused on determining the impact of adding biochar in the fermentation prior to saccharification and yeast inoculation.

2.1. Materials

The milled switchgrass used in this study was described in a previous study (Dien et al., 2006). Enzymes were generously donated by Novozymes North America (Franklin, NC). Retted flax shive was acquired from the USDA Flax-Fiber Pilot Plant (Athens, GA). All chemicals were of research grade and sourced from either Sigma Chemicals or Fisher Scientific. While flax was used as the starting material for the activated char, other similar lignocellulosic waste products may serve the same purpose (Klasson et al., 2011).

2.2. Activated biochar production

Steam activated biochar was prepared from dew-retted flax shive by pyrolysis at 700 °C for 1 h, immediately followed by steam activation at 800 °C for 45 min and was washed before use (Klasson et al., 2011).

2.3. Acid pretreatment and hydrolysis

Seventeen grams of switchgrass was mixed with 80 mL of 1.7% (w/v) H₂SO₄ in 200-mL capped stainless steel vials and heated (while rotating at 60 rpm) at 160 °C for 20 min in an infrared-heater based oven (Labomat BFA-12 v200, Werner Mathis, Concord, NC).

In Study 1, the hydrolyzed liquid was recovered from the solids by filtering with cheese cloth and stored at 4 °C without neutralization.

2.4. Activated biochar treatment of acid hydrolyzate

In Study 1, 10 mL of filtered acid hydrolyzate (~pH 1.1) was combined with various amounts of biochar (0, 0.03, 0.06, 0.1, 0.2, 0.3, 0.4, or 0.5 g) in 40-mL glass vials capped with Teflon™-lined tops and mixed end-over-end for 24 h at ambient temperature. Triplicate experiments were done. After contact, a portion of the supernatant was filtered using 0.45-μm-pore-size syringe filters (Millex-FH, polytetrafluoroethylene, Millipore, Billerica, MA) before analysis (triplicate injections) using HPLC.

2.5. Neutralization and buffering

After acid hydrolysis, the entire content (~100 mL) of the Labomat reactor was neutralized and buffered by adding 7.1 mL of 15% (w/v) Ca(OH)₂ solution and 4.5 mL of 1 M citric acid buffer (pH 4.8) (Dowe and McMillan, 2008). In Study 4, the buffer was changed to 4.5 mL citrate-phosphate buffer (pH 6) (McIlvaine, 1921).

2.6. Enzymatic pretreatment and hydrolysis

In Study 2, filtered and neutralized hydrolyzate (~110 mL) was enzymatically treated by adding 2.7 mL of CTec3 (which contained 37 FPU cellulose/mL and 3900 U xylanase/mL, Novozyme), 2.7 mL of HTec3 (which contained 400 U xylanase/mL, Novozyme), and 320 μL of sodium azide (2.5 mg/L) in capped glass bottles and shaken at 50 °C for 3 days. In Studies 3 and 4, the enzyme mixture was added simultaneously with yeast inoculum.

2.7. Activated biochar treatment of acid/enzyme hydrolyzate

In Study 2, either 5 mL centrifuged neutralized hydrolyzates was combined with biochar (0.15, 0.2, or 0.25 g) or 10 mL of the same hydrolyzate was combined with biochar (0, 0.05, 0.1, or 0.2 g) in 40-mL glass vials with Teflon™-lined caps and mixed end-over-end for 24 h at room temperature. Triplicate experiments were done. After contact, a portion of the supernatant was filtered before analysis (duplicate injections).

2.8. Sequential activated biochar treatment, enzyme hydrolysis, and fermentation

Two experiments were applied. In Study 3, the neutralized and buffered acid hydrolyzate was treated for 2 h with the active char. No sodium azide was added in this case. Without solids separation, the hydrolyzate was inoculated to an OD₆₀₀ of 1.0 with the genetically engineered xylose fermenting yeast *Saccharomyces cerevisiae* strain YRH400 (Hector et al., 2011). Study 4 was conducted in a similar manner with the following modifications: activated char was only pre-contacted for 2 h and the culture was inoculated to an OD₆₀₀ of 3.0. The fermentations were conducted at 32 °C for 5 days. Triplicate control experiments were also treated in the same fashion but without adding activated biochar. In addition, enzyme background fermentations (without hydrolyzates) were also conducted to account for the amount of gas and ethanol produced from sugars originating in the commercial enzyme preparations.

2.9. Fermentation monitoring

Fermentation progress was monitored by measuring exhausted carbon dioxide using gas production modules (Ankom Technologies, Macedon, NY). Carbon dioxide and ethanol productions are stoichiometric. The gas production modules directly measure

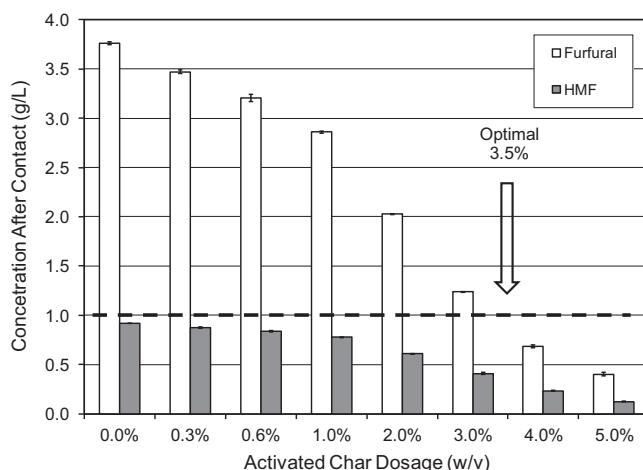


Fig. 1. Furfural and HMF concentration in acid hydrolyzates after contact with activated biochar at various dosing rates. The hydrolyzates also contained 10.7 g/L glucose, 37.4 g/L xylose, 5.9 g/L arabinose, and 6.9 g/L acetate (error bars represent standard deviation of results from triplicate experiments).

cumulative increase in gas pressure, which is converted to mmoles of CO₂ production using the ideal gas law. The gas production modules were set to record gas production every 15 min and to exhaust CO₂ at 6.9 kPa (above ambient).

2.10. Analytical procedures

Furfural and HMF analyses in the first two studies were performed via HPLC (Series 1100, Hewlett Packard/Agilent, Santa Clara, CA) with Zorbax SB-C18 column (3 × 150 mm, 3.5 μm particle size, Agilent, Santa Clara, CA) using a flow rate of 0.4 mL/min at 22 °C. The two mobile phases (A and B) consisted of 95% deionized water with 5% acidified (2.5 g acetic acid/L) methanol and 100% acidified methanol, respectively. The solvent profile was 100% A, 50% B at 15 min, 100% B at 17 min, 100% B at 25 min, 100% A at 28 min, and 100% A at 38 min. The sample volume was 1 μL of filtered hydrolyzates and the diode array detector collected data at a wavelength of 280 nm. Duplicate or triplicate injections were made. Calibration was performed with four furfural and HMF standards. The calibration curve could be approximated with a second order polynomial with shapes similar for both furfural and HMF. In all other studies, sugars, organic acids, furans, and ethanol were measured by injecting 20 μL/sample onto a normal-phase HPLC equipped with a refractive index detector and an organic acid analytical column (Aminex HPX-87H Column, 7.8 × 300 mm, 9 μm particle size, Bio-Rad Laboratories, Inc., Hercules, CA) run at 65 °C at 0.6 mL/min with 5 mM H₂SO₄ (Dien et al., 2004).

3. Results and discussion

Feasibility studies of activated biochar treatment of acid hydrolyzates from switchgrass demonstrated that inhibitor levels could be reduced from 3.8 g/L of furfural and 0.92 g/L of HMF to below a target level of 1 g/L of furfural at a biochar dosage of approximately 3.5% (w/v) (see Fig. 1). Furfural concentrations were observed to be higher than HMF, which is expected because furfural originates from arabinose and xylose liberated by the dilute-acid pretreatment.

In Fig. 2, the adsorption isotherms have been plotted for the experiment. As is noted, because of the higher levels of furfural present, the activated biochar contained higher amounts of furfural than of HMF. In previous studies of furfural and HMF adsorption from sugar solutions, the amount of furans sorbed on to the biochar

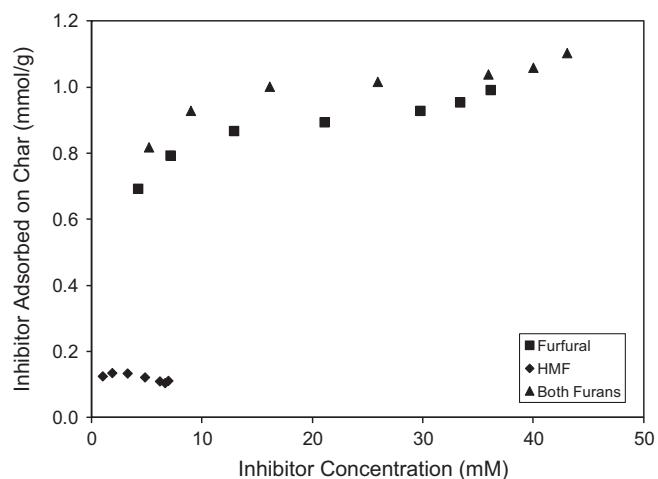


Fig. 2. Isotherm profiles for uptake of furfural and HMF on activated biochar from acid hydrolyzates. Sugar and acid levels are listed in Fig. 1 caption.

corresponded to approximately 1.1–1.3 mmol furan/g of carbon, with the higher value being that for furfural (Klasson et al., 2011). In the present study, the maximum loading was about 1 mmol/g of total furans. The possible reason it was lower in this study, is that there may be a variety of other organics (e.g., phenolics released from partially degraded lignin) in the hydrolyzates that compete for the adsorption sites as compared to a simple sugar solution. Another possible explanation is that hydrolyzate was highly acidic (pH 1.1), while the sugar solution in the previous study was mildly acidic to nearly neutral (Klasson et al., 2011). Other research has suggested that furfural adsorption onto carbon works best at pH 6, or above (Sahu et al., 2008). Other studies have reported furfural uptake capacity of traditional activated carbon to be in the range of 0.24 mmol/g (Sahu et al., 2008) to 3.9 mmol/g (Sulaymon and Ahmed, 2008) under various conditions.

Since the acidic conditions in the acid pretreatment hydrolyzates are considered less than optimal for adsorption (Sahu et al., 2008), further experiments were performed. The concentrations of the furans after contact, with different quantities of activated biochar, in the hydrolyzate that had been neutralized and undergone subsequent enzymatic-mediated hydrolysis as well are shown in Fig. 3. As is noted in the figure, a lower dose of biochar was needed to reduce the furfural to below the goal of 1 g/L. This was likely due to the fact that the concentrations of the furans were lower in these samples. This was anticipated as neutralization of acid hydrolyzates reduces the level of furans (Mussatto and Roberto, 2004).

The isotherms from this set of experiments are presented in Fig. 4 and they look similar to those presented in Fig. 2. Thus, we must conclude that neutralization to a mildly acidic pH (pH 4.5) did not improve uptake (even though less biochar was required), suggesting that other compounds present in the hydrolyzates may bind to some of the adsorption sites. However, this characteristic may not be disadvantageous because lignin breakdown products have also been cited as fermentation inhibitors (Mussatto and Roberto, 2004).

The adsorption of the furans onto the activated biochars was modeled using the following modified Langmuir isotherm equation:

$$q = q_{HMF} + q_{Fur} = \frac{q_{m,HMF} K_{HMF} C_{HMF} + q_{m,Fur} K_{Fur} C_{Fur}}{1 + K_{HMF} C_{HMF} + K_{Fur} C_{Fur}}$$

where q is the sum of furans adsorbed (in mmol/g), q_m is the adsorption limit, C is the equilibrium concentration (in mmol/L, i.e., mM) and K is a equilibrium constant (in L/mmol). The parameters

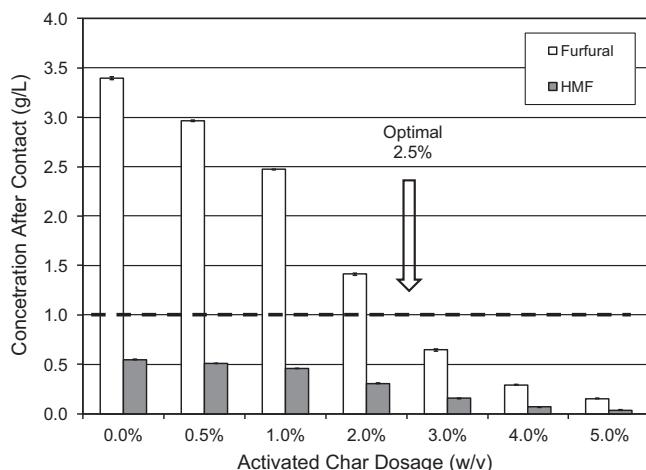


Fig. 3. Furfural and HMF concentration in neutralized hydrolyzates (from acid pretreatment and enzymatic hydrolysis) after contact with activated biochar at various dosing rates. The hydrolyzates also contained 60.1 g/L glucose, 27.5 g/L xylose, 5.2 g/L arabinose, and 5.8 g/L acetate (it is important to note that the enzyme preparations used in the study contributes approximately 15 g/L of glucose and 0.6 g/L of xylose in this liquid. Error bars represent standard deviation of results from triplicate experiments).

were determined for the data sets presented in Figs. 2 and 4 by first forming the ratio q_{HMF}/q_{Fur} and plotting this as a function of C_{HMF}/C_{Fur} and determining the slope of the straight line, representing the $(q_{HMF}K_{HMF})/(q_{Fur}K_{Fur})$ ratio. Once this value was determined, the rest of the variables were determined through nonlinear regression with bi-square weighting of the data according to the procedure described by Duggibley (1981). The result of the modeling can be seen in Fig. 5, where the uptake of both furans has been plotted as a function of the sum of the furan concentrations in the liquid phase at equilibrium.

The effect of activated biochar (ABC) addition to the fermentation broth (after neutralization but before enzyme hydrolysis and fermentation) is shown in Fig. 6. It is notable that the yeast fermentations completely failed without biochar addition. In contrast, biochar addition resulted in a vigorous fermentation, but only after an 80-h long lag phase and only in two of the three replicates. This finding suggested that the yeast were still inhibited to some extent in the biochar-amended fermentation vessels. Extended lag phases

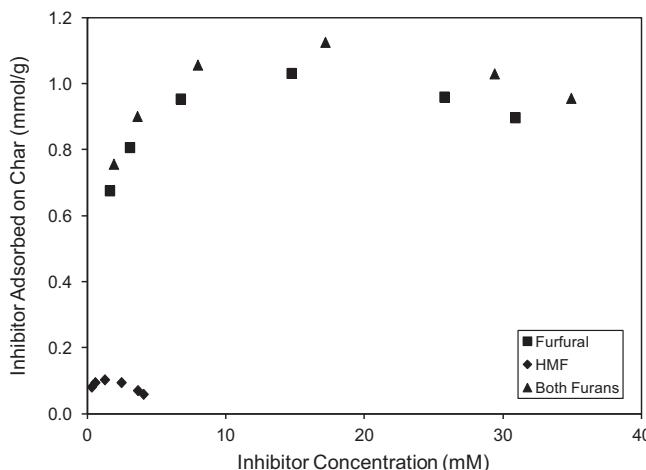


Fig. 4. Isotherm profiles for uptake of furfural and HMF on activated biochar from acid hydrolyzates which had been neutralized and treated by enzymes to solubilize all sugars. Concentration of sugars and acid in the hydrolyzates was 60.1 g/L glucose, 27.5 g/L xylose, 5.2 g/L arabinose, and 5.8 g/L acetate.

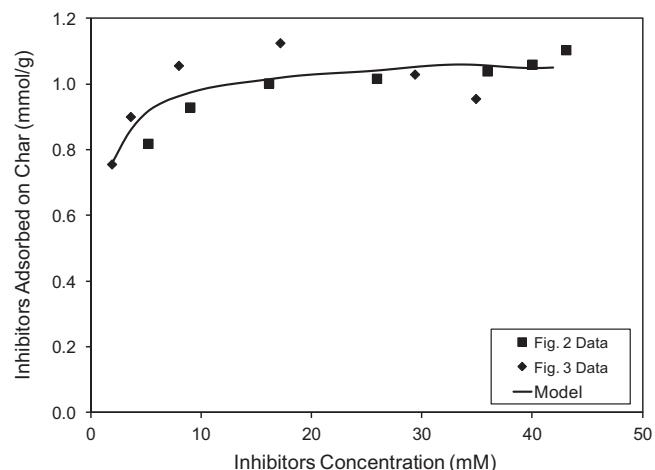


Fig. 5. Comparison of the modified Langmuir isotherm with the raw data. The fitted constants in the Langmuir isotherm equation were: $q_{m,HMF} = 0.65 \text{ mmol/g}$, $K_{HMF} = 1.35 \text{ mmol/L}$, $q_{m,Fur} = 1.16 \text{ mmol/g}$, and $K_{Fur} = 1.22 \text{ mmol/L}$.

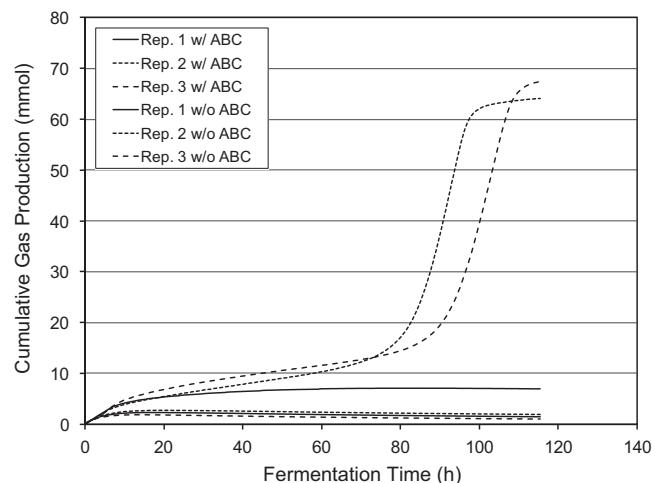


Fig. 6. Effect of activated biochar addition to neutralized acid hydrolyzate 24 h prior to enzymatic hydrolysis and fermentation.

are a commonly observed symptom of hydrolyzate-associated inhibition.

The furfural, HMF, and sugars concentrations are listed in Table 1. The concentration of furfural in the untreated hydrolyzates was 4.3–4.4 g/L (45–46 mM) and 1.4–1.7 g/L (15–18 mM) in the treated samples. The treated reactor that did not exhibit any gas production contained the lowest beginning concentration (1.4 g/L) of furfural. We have observed similar inconsistent results (e.g., 2 out of 3 fermentations working) when furan concentrations are near the maximum tolerance of yeast (data not shown). The composition of the hydrolyzates following fermentation is listed in Table 2 (if comparing Tables 1 and 2, note that concentrations measured

Table 1

Concentration of main constituents before enzymatic hydrolysis and fermentation in biochar treated and untreated hydrolyzates.

	ABC treated			Not ABC treated		
	Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3
Furfural (g/L)	1.4	1.7	1.7	4.3	4.3	4.4
HMF (g/L)	0.3	0.4	0.3	0.7	0.7	0.7
Glucose (g/L)	9.9	9.6	10.2	10.4	10.4	10.3
Xylose (g/L)	26.8	27.1	26.7	27.2	28.1	27.8
Arabinose (g/L)	4.8	4.8	4.9	5.0	5.1	5.1

Table 2

Concentration of furans and sugars after fermentation in biochar-amended and unamended fermentations.

	ABC treated			Not ABC treated		
	Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3
Furfural (g/L)	0.8	0.0	0.0	3.0	2.9	3.2
HMF (g/L)	0.3	0.1	0.1	0.5	0.5	0.5
Glucose (g/L)	48.3	0.2	0.3	58.3	58.4	57.3
Xylose (g/L)	24.2	22.2	22.4	25.0	25.2	24.8
Arabinose (g/L)	3.8	3.8	3.9	3.9	3.9	4.1
Ethanol (g/L)	3.8	30.1	30.9	1.1	1.3	0.9

after fermentation represent values in a slightly larger volume due to inoculation). The presence of glucose in the failed fermentations is evidence that the cellulases were functioning and the failure can be attributed to the yeast. What is also noted is that the furfural and HMF concentration after the fermentation is significantly reduced in reactors with active fermentation and it has been shown that yeast have the capability to deplete furans given enough time (Boyer et al., 1992). When presented with furans, the aldehydes are biologically transformed to their alcohol form by the action of alcohol dehydrogenase. It is not unexpected that the first biochar trial fermentation failed because furfural remained at the end of the run. Unfortunately, xylose was not consumed in any of the fermentations. YRH400 ferments xylose much slower than glucose (Hector et al., 2011). Furthermore, while glucose fermentation is somewhat tolerant to higher acetic acid levels, xylose fermentation by *S. cerevisiae* has been shown to be significantly impaired at acetic acid concentrations as low as 1 g/L, especially at lower pH (Casey et al., 2010; Wright et al., 2011).

While promising results were obtained for the simultaneous saccharification and fermentation of biochar-amended hydrolyzates, the long fermentation lag phase is not commercially viable because it affords opportunity for contamination and lowers productivity. To optimize the process, the inoculum size and the pH of the fermentation were increased. High inoculum size has been shown to be a very effective method of improving fermentability of furan-containing hydrolysate (Boyer et al., 1992). It is also known that acetic acid, here originating from the hemicellulose, is a potent fermentation inhibitor. However, the effect of acetic acid can be partially abated by raising the pH above that of the pK_a (4.76) (Mussatto and Roberto, 2004). Increasing inoculum and raising pH should also promote xylose metabolism (Casey et al., 2010). Furthermore, the long treatment time (24 h) of the neutralized hydrolysates with the activated biochar added significant time to the overall process. In order to address the latter of the concerns, a kinetic study was conducted where the centrifuged neutralized (and enzymatically treated) hydrolysates was contacted with activated biochar for different times. The results show (Fig. 7) that the majority of furfural and HMF adsorption took place within 60–120 min. This result is similar to that obtained by Lee et al. (2011) where it was found that activated carbon acted as quickly as in 1 h, removing 92–93% of the inhibitors, using activated carbon charcoal (2.5%, w/v).

The results of fermentation under improved conditions are shown in Fig. 8, where the fermentation pH was increased from 4.7–5.1 to 5.2–5.5, the inoculum size was increased by a factor of three, and the contact time with activated char was reduced from 24 to 2 h. It is apparent that the fermentation in the biochar-amended cultures progressed very well, giving very consistent results among the triplicate bioreactors. This was not the case with the fermentation in the non-biochar-amended cultures, where each of the cultures showed prolonged lag phases, albeit to varying extents. It is notable, in contrast to results observed from Study 3 (Fig. 6), the untreated control fermentations did not stall

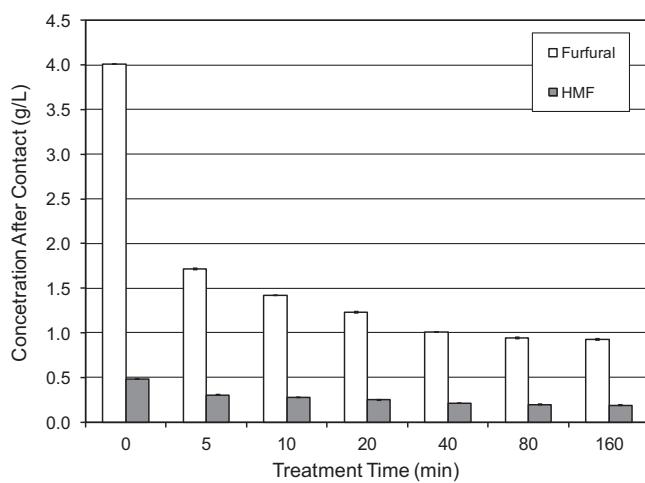


Fig. 7. Determination of treatment time required to treat neutralized acid hydrolysis (error bars represent standard deviation of results from duplicate experiments).

completely, suggesting that increasing the inoculum size and pH did indeed help the yeast to cope with presence of fermentation inhibitors. Still, the reduced production rate (from cell growth lag) observed (Fig. 8) are beyond what is commercially acceptable because of the increased dangers of microbial contamination. The amount of activated char added in these experiments is similar to those proposed by others. Mussatto and Roberto (2001) noted a 27% removal of phenolics by activated charcoal (2.5%, w/w) and improved fermentation yields; it should be noted that the activated charcoal was removed before fermentation. Likewise, Alves et al. (1998) found optimal fermentation yield when sugarcane bagasse hydrolysate was treated with 2.4% of activated charcoal, but again the charcoal was removed before the fermentation.

The main constituents present in the broth before and after simultaneous enzyme hydrolysis and fermentation are shown in Tables 3 and 4 (if comparing Tables 3 and 4, note that concentrations measured after fermentation represent values in a slightly larger volume due to inoculation). Modifying the culture conditions did encourage use of xylose; 40–50% of the xylose was consumed in the biochar-amended cultures. Little if any xylose was consumed in the untreated controls. Unfortunately, it does not appear that xylose was fermented to ethanol—ethanol concentrations were 32.5 ± 1.2 g/L and 31.4 ± 1.7 g/L for treated and untreated, respectively. Instead, what xylose was consumed by the

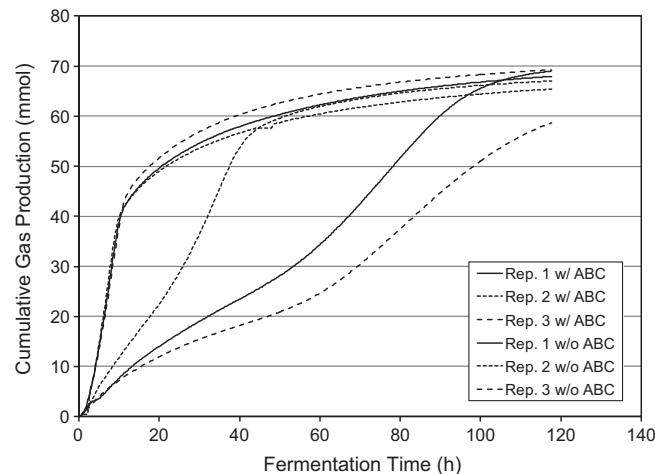


Fig. 8. Fermentation activity of biochar-amended and un-amended hydrolysates at higher inoculum levels.

Table 3

Concentration of main constituents before enzymatic hydrolysis and fermentation in biochar treated and untreated hydrolyzates.

	ABC treated			Not ABC treated		
	Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3
Furfural (g/L)	0.8	0.6	0.7	2.8	2.7	3.0
HMF (g/L)	0.3	0.2	0.2	0.7	0.7	0.7
Glucose (g/L)	9.3	9.6	9.5	9.6	10.0	9.5
Xylose (g/L)	31.3	32.0	31.1	31.2	31.3	31.1
Arabinose (g/L)	5.1	5.1	5.1	5.1	5.1	5.1

Table 4

Concentration of main constituents after fermentation in biochar-amended and unamended fermentations with increased inoculum size.

	ABC treated			Not ABC treated		
	Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3
Furfural (g/L)	0.0	0.0	0.0	0.0	0.0	0.0
HMF (g/L)	0.1	0.1	0.1	0.1	0.1	0.1
Glucose (g/L)	0.5	0.8	0.7	1.4	1.2	4.7
Xylose (g/L)	16.4	19.5	19.8	26.7	25.4	27.5
Arabinose (g/L)	3.8	4.0	4.0	4.1	4.2	4.2
Ethanol (g/L)	32.7	31.2	33.6	32.1	32.6	29.5

yeast was converted to xylitol (6–8 g/L). Xylitol is the first intermediate in the xylose fermentation pathway and has been observed to be the dominant side-product for this strain as well as other *Saccharomyces* engineered for xylose metabolism (Hector et al., 2011). Production of xylitol suggests a bottleneck downstream in the xylose-fermentation pathway and/or a redox imbalance. The final concentration of ethanol was approximately the same in all the bioreactors, but the fermentation started right away and proceeded much faster in biochar-amended bioreactors than those of the control.

While the impact of activated biochar on sugar availability was not studied in this research, previous work with activated carbon showed that only small amounts (~9%) of sugars are removed by the carbon (Lee et al., 2011), and it is important to note that, since in our experiments the biochar material remains in the broth during fermentation, the sugars are presumably partially available for consumption. Acetate levels in biochar-amended and un-amended fermentation did not appear to be significantly different (5.5–6.3 g/L) and thus, did not impact the glucose fermentation to any extent. The acetate inhibition of xylose fermentation can be overcome by acclimating yeast to acetate (Wright et al., 2011).

4. Conclusion

Activated biochar made from flax shive was successfully used to adsorb the fermentation inhibitors furfural and hydroxymethyl-furfural from switchgrass acid hydrolysis. Rate studies showed that the majority of the inhibitors could be reduced in as little as 1–2 h. Enzyme hydrolysis and simultaneous fermentation performed in biochar-containing hydrolyzates showed that the fermentation progressed very well if the furfural had been reduced sufficiently. Fermentation was still possible in hydrolyzate containing approximately 1.5 g/L furfural but long lag phases were observed and inconsistency between replicate fermentations. Increased inocula levels overcame some of the inhibitory effects in fermentation of non-biochar amended hydrolyzates but the results were not repeatable and reduced fermentation rates were noted.

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